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Conference Report

Sperm-mediated gene transfer: advances in sperm cell research and applications

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A meeting entitled 'Sperm-mediated gene transfer: advances in sperm cell research and applications' was held on May 23–26, 1999 at the University of Siena, Certosa di Pontignano, Siena, Italy. The irony of holding a meeting about sperm and transgenic animals in a spectacular 15th century monastery was not lost on the participants. Profs Baccio Baccetti, Instituto di Biologia Generale, Siena and Corrado Spadafora, Instituto Tecnologie Biomediche, CNR, Rome organized the meeting with able help of Drs Roberto Giordano and Anna Rosa Magnano, both of Siena. Twenty-two speakers from 12 countries shared their insights into basic sperm biology and experiences producing transgenics by sperm-mediated gene transfer. The two days of presentations were equally balanced between the basic and the applied.

The first day's talks were devoted to basic sperm biology, especially as it related to DNA packaging and expression of the sperm genome. The first talk given by Rod Balhorn from Lawrence Livermore National Laboratory in California set the tone for a truly dynamic series of presentations. Dr Balhorn concluded his talk of protamine DNA interactions by showing a video of his work with atomic force microscopy. He literally dragged strands of DNA around to demonstrate how exposing naked DNA to protamines almost instantly altered the configuration of the long linear strand of DNA into tightly condensed knots (toroidal DNA condensates). In the second talk, Dr Steven Ward (Robert Wood Johnson Medical School, New Jersey) discussed the sperm genome and its interaction with the sperm nuclear matrix. He presented evidence for sperm DNA loop-like structures that are associated with the nuclear matrix. Both of these talks made it clear that even though sperm DNA is, for the most part very tightly packaged in the sperm nucleus, there are exposed segments of the genome which may possibly serve as sites for integration of exogenous DNA.

A subsequent series of interesting talks focused on spermatogenesis and expression of the haploid genome by Drs Hecht, University of Pennsylvania, Krawetz, Wayne State University and Miller, University of Leeds.

Probably the talk that best unified basic sperm biology with sperm-mediated gene transfer was presented by Corrado Spadafora. Dr Spadafora presented compelling evidence for a model of sperm-DNA interaction that seems to explain much of what has been observed when sperm is exposed to exogenous DNA. He has published at least some of his model which describes an active transport (energy requiring) of exogenous DNA into sperm and for the involvement of CD4 in initiating an apoptosis cascade with a concomitant release of nucleases from the sperm (BioEssays 20: 955–964, 1998). His model accounts for the observation that high concentrations of exogenous DNA can kill sperm and for the apparent rearrangement of transgenes introduced by sperm-mediated gene transfer. Dr Spadafora reported that sperm from different males vary in their responsiveness to exogenous DNA. Triggering apoptosis and the consequential release of nucleases requires higher DNA concentrations for sperm from some males (see below). Corrado also showed that a protein in seminal plasma, he calls IF-1, blocks the uptake of exogenous DNA by sperm. That apparently accounts for the observation that one must remove seminal plasma as part of the sperm-mediated gene transfer protocol. In addition, he presented evidence demonstrating that the incoming DNA binds the sperm nuclear matrix apparently prior to becoming integrated into the sperm genome.

The applied talks covered silkworms, shellfish, fish, frogs, rabbits, chickens, mice and cattle. Generally, but there were exceptions, transgenes can be successfully transferred into founders via sperm. In silkworms, Dr Sinnakaruppan Mathavan, Madurai Kamaraj University, India reported that transgenics

can be produced by injecting transgenes into the testis of the silkworm prior to mating. In other species mature sperm exposed to exogenous DNA were then used for either *in vitro* or *in vivo* insemination. Often transgenes appear to be rearranged, negating the possibility of expression in most cases. There seems to be a high proportion of germline mosaic founders and it was not uncommon for transgenes to disappear in subsequent generations. There was a lot of talk about the possibility of episomal transgenes, but no direct evidence was presented. However, there seemed to be two glaring exceptions to these general findings.

The first involved transgenic chickens were reported by Dr Mordechai Shemesh of the Kimron Veterinary Institute, Israel. He bounded transgenes to liposomes before exposing the DNA to sperm. That treatment did not yield transgenics but when he also added restriction enzymes to the mix he reported the successful production of transgenic chicks. The study is a work in progress and as a consequence no direct molecular evidence (e.g., Southern or northern data) was presented which would have helped in interpretation of his findings. He did share some indirect expression data that seemed consistent with what one would have expected if the procedure worked properly.

The other example that clearly demonstrated exposing sperm to exogenous DNA could result in functional transgenic animals was a report by Anthony Perry, University of Hawaii School of Medicine. In the final talk of the meeting Dr Perry demonstrated that nuclei from membrane damaged sperm could carry transgenes into oocytes via intra-cytoplasmic sperm injection (ICSI; Science 284: 1180–1183, 1999). His data were solid and very impressive (the brightly glowing GFP-expressing transgenic mouse pup was quite surrealistic). There was little doubt he was making transgenic mice by his method and that the transgenes behaved as expected (some founders expressed the transgene and transgene inheritance was approximately Mendelian). Dr Perry proposed that by his methodology transgenes were not necessarily integrated into the sperm genome prior to transport by sperm into the oocyte. This concept stimulated a lively conversation.

Both of Drs Shemesh's and Perry's reports seemed to support the concepts embodied in Spadafora's model. The liposomes and ICSI both provide a means of bypassing the apparent membrane mediated trigger to apoptosis.

There were adequate data presented at the meeting to support the notion that exogenous DNA can be bound by sperm and that at least a proportion of that DNA can be transported into oocytes. Furthermore, the exogenous DNA can be detected in some of the offspring produced. Dr Spadafora's model is supported by his own work and that of others and seems like a useful point of departure for testing hypotheses designed to explain the mechanism by which sperm-mediated gene transfer operates.

After the stimulating two day meeting I also had an opportunity to visit Marialuisa Lavitrano's laboratory in Rome. She, as you will recall, was the first author on the Cell paper who started this modern version of the field (remember Ben Brackett reported sperm-mediated gene transfer in Proc. Natl. Acad. Sci., USA 68: 353–357, 1971, as usual being far ahead of the times). Dr Lavitrano has produced an impressive number of pigs by this technique and the immunofluorescence expression data (of Decay Accelerating Factor, DAF) were most impressive. She also had southern and northern blots that supported her contention that her pigs were indeed transgenic. Dr Lavitrano is preparing a manuscript describing this project and has presented preliminary findings this year (Lavitrano, et al., Transplantation Proceedings, 31: 972–974, 1999). The question was raised as to how Dr Lavitrano was able to produce functional transgenic mammals by classical sperm-mediated gene transfer (i.e., wash sperm, add DNA, then inseminate) where others get rearranged transgenes or no transgenics at all. It is her contention that the reason she is having success is because she carefully screens sperm donors before using them for sperm-mediated gene transfer. She has published the concept that sperm from different males differ in their sensitivity to exogenous DNA. DNA concentrations that trigger apoptosis and nuclease releasing events in the sperm of some males have little affect on the sperm from other males. So, according to Dr Lavitrano, you must first identify sperm, a high proportion of which bind DNA at relatively low DNA concentrations. Males that produce such sperm are considered good candidates for sperm-mediated gene transfer. This may not be good news for those using the transgenic mouse model. It is difficult to conceive of a practical method for screening sperm from an individual male without destroying the source. Dr Lavitrano further proposes that there is a specific window of opportunity, during the early stage of capacitation, that is optimum for exposing sperm to exogenous DNA. Her suppositions and observations seem to fit the Spadafora model.

This field deserves watching. That is especially true for those of us dealing with large animals if a data set I saw showing over 50% transgenic animals born (about half with functional transgenes) holds up to replication in other labs. If (now I am leaning towards when) the unresolved issues such as prevalence of germline mosaicism and episomal vs. integrated transgenes can be addressed in a practical way, sperm-mediated gene transfer offers an appealing alternative to the current strategies of producing transgenic animals.